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14. ABSTRACT

Tumor tissue is made up of tumor cells and other surrounding cell types in its microenvironment. This diseased tissue frequently exists in specialized tumor microenvironment such as being less oxygenated and more acidic than healthy tissue. These states profoundly impact anti-tumor drug efficacy. For disease diagnosis, a tumor is frequently biopsied. We believe that if we can maintain a biopsied portion of the tumor tissue in a similar tumor microenvironment, that we can preserve the exact same tumor cellular functions ex vivo. The objective of this project is to develop biomicroelectromechanical systems (BioMEMs), the Breast Cancer Tissue BioReactor that will support the combination of the 3-D culture methodology with control over microenvironmental oxygen and matrix stiffness to better approximate the in vivo tumor microenvironment ex vivo. This setting then would allow testing of anti-tumor drug responsiveness using a patient's own tumor tissue targeted with anti-drug therapeutics. We will test our BCTB using mouse models of breast cancer.

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Bioreactor, tumor microenvironment, breast cancer, microfluidics

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INTRODUCTION:

Tumor tissue is made up of tumor cells and other surrounding cell types within its microenvironment(1-4). Furthermore, this diseased tissue frequently exists in a less oxygenated (termed "hypoxic") environment than does healthy tissue(5-8). This unique state selects for cell populations that thrive in this harsh climate. All of these states profoundly impact anti-tumor drug efficacy(9-13). Frequently, tumor biopsies are used to assess the state and progression of the disease. We believe that if we can maintain a biopsied portion of the tumor tissue in a similar tumor microenvironment, that we can preserve the exact same tumor cellular functions ex vivo. This setting then would allow testing of anti-tumor drug responsiveness using a patients own tumor tissue targeted with anti-drug therapeutics. The objective of this project is to develop biomicroelectromechanical systems (BioMEMs) that will support the combination of the 3-D culture methodology with control over microenvironmental oxygen and matrix stiffness to better approximate the *in vivo* tumor microenvironment *ex vivo*. We are fabricating the proposed BioMEMS devices and the sensor arrays used to substantiate the base oxygen and pH gradients using soft lithography and thin film deposition techniques. Matrix tension in being altered by using defined ECM compositions to generate patterns of matrix stiffness. These devices and capabilities are currently being validated using traditional normal and tumorigenic mammary epithelial cell culture systems grown under 3D cell culture conditions that allow for mammosphere or glandular mammary acinar development. Drug delivery into the device has been validated using a Protease inhibitor compound that can inhibit mammosphere formation within the BCTB. Xenografts generated in mice will be used for biopsy samples to validate the maintenance of the *in vivo* tumor cellular functions *ex vivo* in the Breast Cancer Tissue BioReactor. Once these base line conditions are established, we will correlate the drug response of the tumor biopsy challenged within the Breast Cancer Tissue BioReactor with the corresponding outcome *in vivo* against xenograft models of the same.

BODY:

Breast Cancer Tissue Bioreactor for Direct Interrogation and Observation of Response to Anti-Tumor Therapies.

Our work this year focused on 3 key areas: 1) validating drug delivery in the system; 2) continued development of the Breast Cancer Thick-tissue Bioreactor to include controlled oxygen and pH environments with simultaneous measurements using thin film sensors; and 3) testing of various sterilization techniques and subsequent thin film performance and mechanical integrity. In combining oxygen, pH and matrix stiffness, our design features delivery of gas (i.e., oxygen and nitrogen) <u>through</u> PDMS. Thus, determining the parameters controlling diffusion of gas through PDMS with various treatments (i.e., surface treatments, increasing cross-linking of PDMS) was essential for the design features. Regarding item #3, we had an unanticipated hurdle this year. As we developed the thin films and moved to apply this tool with long-term cell culture, we began to sterilize with our standard techniques and had a difficulty with maintaining sample integrity. We have tested a variety of conditions and feel we have a good sterilization protocol currently and we are currently testing our thin films for oxygenation analysis with mammospheres. This is described in detail below.

Task 1: To Develop the Breast Cancer Tissue Bioreactor (BCTB) and functionalize with controlled matrix rigidity in the surrounding microenvironment.

A) Develop and fabricate high throughput Breast Cancer Tissue Bioreactor (BCTB) disposable cartridges.

The Breast Cancer Tissue Bioreactors (BCTB) were fabricated in PDMS using standard soft lithography and replica molding techniques. The device consists of two parts: a 1 mm thick PDMS membrane that contains four 3 mm in diameter Cell/Biopsy growth chambers (separated by 5.85 mm) and a top 4 mm thick PDMS piece that contains the microfluidic supply networks for each chamber (Figure 1A and B). The membrane is bonded to a 2" x 3", 1 mm thick glass slide to provide structural support and to allow cell imaging through an inverted microscope. Growth chambers are in direct contact with supply networks or are separated from microfluidic supply channels by a polycarbonate filter (Osmonics, Inc) with 0.4 micrometer pores, that maintains diffusional exchange of nutrients and waste products and prevents leakage of ECM into the microfluidic channels. The supply network for each of the cell growth chambers consists of 8 microfluidic channels 100 µm wide x 100 µm tall with 100 µm walls connected together to a larger 800 µm wide input / output regions of the network (Figure 1A and B). The entire reactor is clamped together under sterile conditions in a custom built polycarbonate and stainless steel clamp that allows for easy alignment of the reactor components, provides structural stability to the assembly, and evenly distributes holding pressure across the PDMS surfaces (Figure 1C and D). All 4 individual reactors in a single BCTB cartridge were gravity fed from a modified 14 mL tube with a loose fitting cap (i.e. a "snap cap tube") mounted in a custom built holder. The output of the feeding tube was directly connected to a 50 µm tall microfluidic manifold splitting the input flow into 4 even streams for each of the reactor within the cartridge. A second output manifold (on the waste side of the reactor) was used to passively regulate the flow rate as described in detail below; to recombine the outflow from 4 reactors; and, to direct it into a waste collection ("Labtainer" ™ from HyClone) or sample extraction systems (Figure 1D and E). The fluid

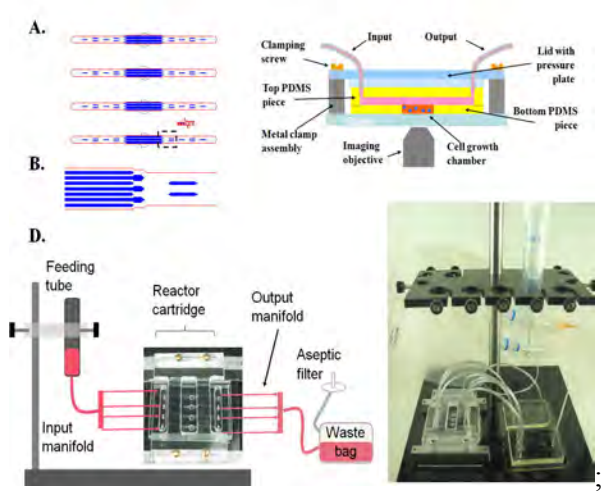

Figure 1. <u>Principle design of the Thick Tissue Bioreactor (TTB).</u> A) AutoCAD drawing of the lithographic mask for TTB microfluidic channels, dotted circles indicate cell culture chambers; B) A close view of the binary flow divider with ceiling support pillars; C) Block-diagram of the typical layout for the clamped TTB indicating major components; D) A photo of the clamped TTB cartridge with a diagram illustrating set up for long term culture; E) A photo of the fully assembled TTB prototype with the gravity driven feeding system, passively regulated flow through the input/output manifold, and a "Labtainer" ™ from HyClone as a waste containment system.

levels in the feeding tube were always held at 15 cm above the surface. All tubing was 0.020" ID, 0.060" OD Saint-Gobain* Tygon* microbore tubing (Part # AAQ04103) from Fisher Scientific.

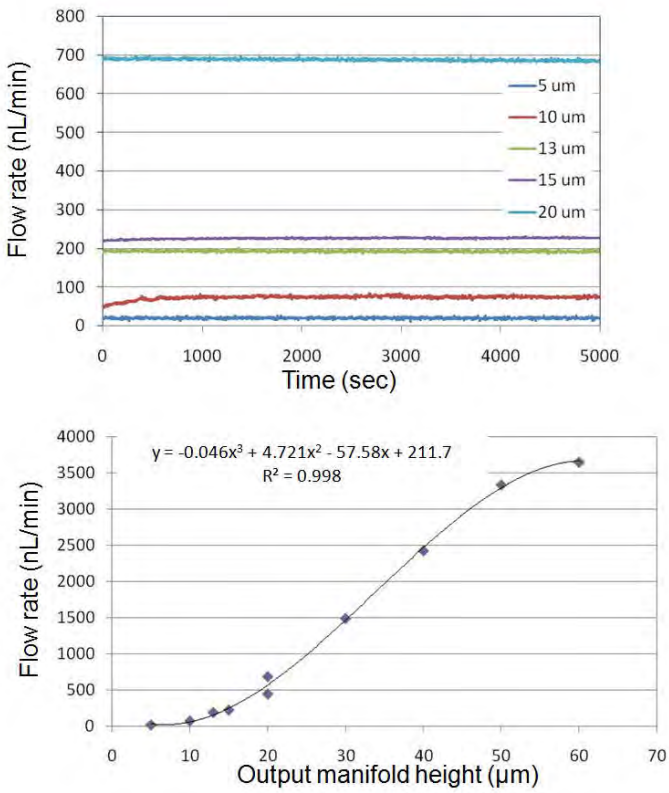

Figure 2. <u>Effect of the output manifold height on the total flow rate through TTB.</u> A) Typical flow rates through BCTB with the output manifolds of varying heights. B) A photo of the BCTB connected to the input and output manifolds. C) Flow rate as a function of output manifold height. Each point is a 20 minute average with error bars representing standard deviations. Upchurch Nanoflow sensor was used for the flow measurements.

In order to achieve targeted media exchange rates within the BCTB, we compared the flow rates using a variety of differentially sized manifolds. Each individual channel in the input manifold was 100 µm wide and 50 µm tall. The channels in the output manifold were 100 µm wide but their height was varied between 5 and 60 µm. The flow rates through the assembled system for each of the output manifold height were measure using an Nanoflow Sensor (Upchurch). By incrementally adjusting the output manifold dimensions, we were able achieve a variety of media flow rates between 20 and 3500 nl/min (Figure 2). Our target flow rate of 700 nL/min (1 mL/day) was achieved with the output manifold height being 20 µm (maintaining channel width of 100 µm). Additionally, we found that the 5 µm manifold was difficult to "wet" and load with media once assembled. In addition, these sizes are of such a small dimension, that any stray particular dust would potentially block a channel. Thus, we do primarily assembly of the devices within clean rooms.

B) Establish BCTB with varying matrix stiffness . We will test the following methodologies in order:

i) Varying concentration Matrigel.

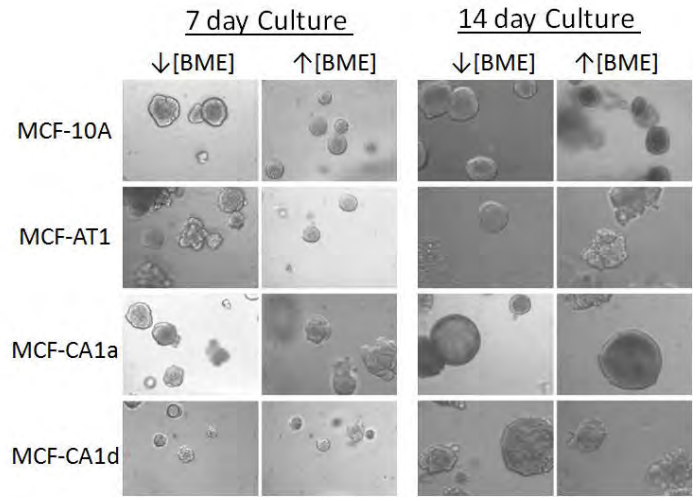

Figure 3. MCF-10A, -AT1, -CA1a and -10 CA1d cell line variants were cultured in the presence of standard (~3 mg/ml Matrigel) or High Concentration Basement Membrane Extracts (20 mg/ml). Shown are representative images of mammosphere formation at 7 and 14 days of culture.

As a test case for suitability of this system to maintain tissue viability, we previously compared mammosphere formation of MCF-10A cell lines and their variants in a chamber slide culture system (Nunc) that utilizes a thin slide compatible with confocal microscopy in parallel with cells cultured within our BCTB. In both cases cell chambers were coated with thin layer of Matrigel. Cells are resuspended in Matrigel ($3x10^5$ cells/ml) and plated into 8-well cell chamber (~70 µl/well) or bioreactor cell chamber (~7 µl/well) and allowed to gel for 1 hr at 37°C. Note that for the same resulting thickness of 3D gelled Matrigel in both systems, we are able to reduce our reagents consumption by a factor of 10 in the bioreactor as compared to the chamber slide. Cells are maintained in Growth Media consisting of DMEM/F12, 5% Horse Serum, 0.1 µg/ml insulin, 0.5 µg/ml hydrocortisone , 0.1 µg/ml cholera toxin, and 20 ng/ml EGF DMEM/F12, 5% Horse Serum, 0.1 µg/ml insulin, 0.5 µg/ml hydrocortisone , 0.1 µg/ml cholera toxin, and 20 ng/ml EGF. Cell cultures are maintained by either media changes every 3 days (8-well slide chamber) or with continuous perfusion in bioreactor.

We used this same system to begin applying variations in extracellular matrix composition within the BCTB. We are targeting "normal" and "high" specialized basement membrane ECM densities. We used our MCF10A and variant lines grown under organotypic conditions and compared mammosphere formation at 2 defined Matrigel/Basement membrane extracellular matrix densities. Cells were seeded within 3D matrigel at low (3 mg/ml) or high (20 mg/ml) BME concentrations. Mammosphere formation was analyzed over time. This will be used to standardize conditions within our BCTB culture system.

Task 2. To Develop Breast Cancer Tissue Bioreactor (BCTB) with controlled oxygen and pH in the surrounding microenvironment.

A) Adapt BCTB to include control of normoxic to hypoxic conditions within same BCTB cartridge. (M 1-14)

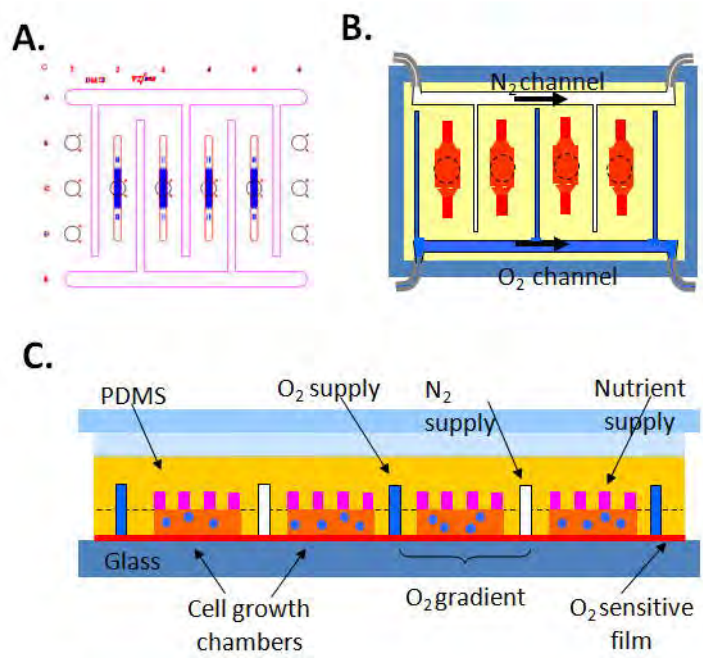

Figure 4. <u>Oxygen Gradient Formation</u>. A) Multilayer photolithographic masks for fabrication of modified BCTB with oxygen delivery network. BCTB with individually addressable gas channels allowing capability of maintaining each cell-culture chamber at distinct oxygen concentrations. Black circles illustrate cell-culture chambers, pink – gas delivery channels (oxygen sources and sinks), red and blue – nutrient delivery microfluidic channels. B,C) A scheme for O_2 delivery inside BCTB. Source and sink channels contain proper mixture of O_2 and N_2 to achieve target (normoxic / hypoxic) conditions.

i) Specific O_2 concentration will be established for each of the biopsy / cell chambers within the same BCTB cartridge, where each of compartment will experience a similar O_2 concentration (i.e. , normoxic or hypoxic).

We have designed several variations of the Breast Cancer tissue bioreactor cartridge with oxygen supply and drain channels to establish particular normoxic or hypoxic conditions. One design features single channels for both oxygen supply and sink with interdigitated blind extensions that provide a fixed oxygen concentration for all of the cell culture chambers within the BCTB cartridge shown in Figure 4. This is the format for providing defined oxygen concentrations within the BCTB that we are evaluating.

ii) Optimize 2-dimensional detection and quantification of O2 based on O2 sensitive thin film.

Oxygen sensitive compound Platinum(II)-tetrakis(pentaflourophenyl)porphyrin (Pt-TPFP) immobilized within a thin (~1.5 μm thick) polystyrene matrix spun on top of a standard microscope glass slide was used as an oxygen sensor(14;15). It was found that adhesion of such film to a microscope slide in the wet environment of cell culture was not consistent. The adhesion was drastically improved if the glass slides were pre-coated with 1% solution of polyvinyl alcohol before depositing a 10% w:v solution of polystyrene in toluene containing Pt-TPFP in 1:100 w:w to PS. As oxygen diffused through the 1.5 μm thick PS film it interacted with the Pt-TPFP immobilized within the film quenching its fluorescence. Fluorescence images were taken with Zeiss microscope Axiovers 25 equipped with QColor 5 cooled ccd camera, and Tx Red fluorescence filter set. Before each experiment film performance was calibrated with 0%, 21%, and 100% oxygen and Stern-Volmer relationship was used to determine quenching constant Ksv.

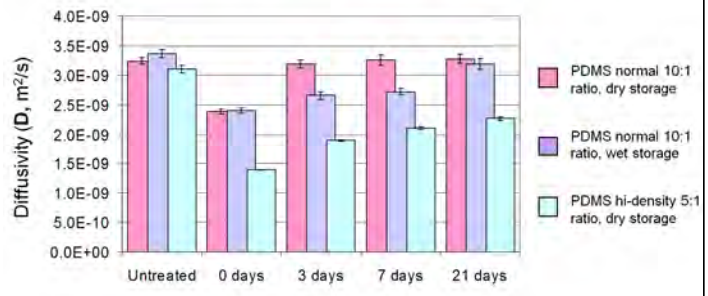

Figure 5. <u>Experimentally determined oxygen diffusivity through 1 mm thick PDMS membranes</u> as a function of surface properties and storage conditions. Note: 1) normal and high density PDMS have almost identical O_2 diffusivities in native state; 2) initial plasma treatment significantly decreases O_2 diffusivity, in some cases by more that 50%; 3) original diffusivity values are fully restored in 3 days for normal PDMS stored in air, while hi-density PDMS does not recover in 3 weeks under the same storage conditions; storage under water (normal operating conditions for a bioreactor) delays recovery by 3 weeks. Error bars represent the 95% confidence interval based on 3 independent measurements with 3 different membranes.

During our preliminary experiments, we noted that the time it took for oxygen to be delivered to the cell culture chamber varied significantly with age of our BCTB cartridges. In order to estimate time evolution of oxygen gradients, the effect of PDMS treatments with oxygen plasma and consecutive aging of the surface, we fabricated a series of PDMS membranes, subjected them to various treatments and determined diffusion coefficients of oxygen across them. We have continued our testing until we could achieve significance to validate our finding. In order to determine oxygen diffusion coefficients, the treated PDMS membranes were placed on top of the Pt-TPFP oxygen sensitive film and clamped with a Plexiglas lid containing a gas delivery channel. Using the Stern-Volmer relationship and Fick's Law of simple diffusion, the diffusivity of oxygen in PDMS was measured for the untreated, freshly treated, and aged membranes for normal (10:1) and more dense (5:1) PDMS formulations. It was noticed that plasma treatment of PDMS prior the reactor assembly reduces the effective diffusion coefficient by 50% thus increasing time required to for the oxygen to get to the cell culture chamber. However, with time the silica formed on the surface is destroyed and the gas diffusion is restored to the untreated levels within three days for regular PDMS formulations, but as it can be seen

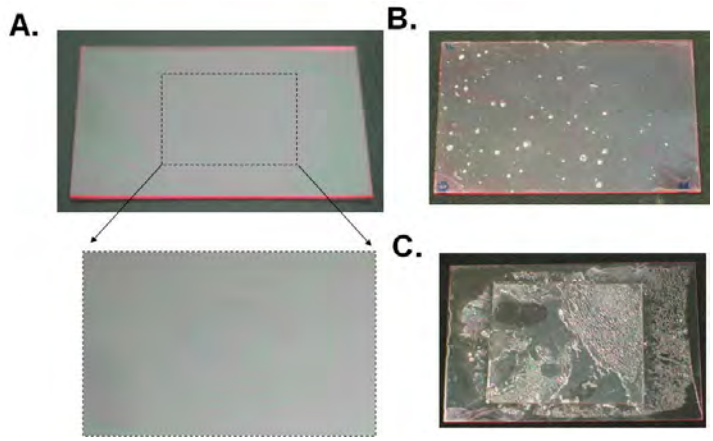

Figure 6. <u>Mechanical failure of the O_2 sensitive film after a standard 20 min sterilization cycle in the autoclave.</u> A) Freshly fabricated thin film. B) Film failure following autoclaving. C) Film failure following autoclaving with the 1 mm thick PDMS slab placed on top of the film to mimic presence of the BCTB cell culture chambers.

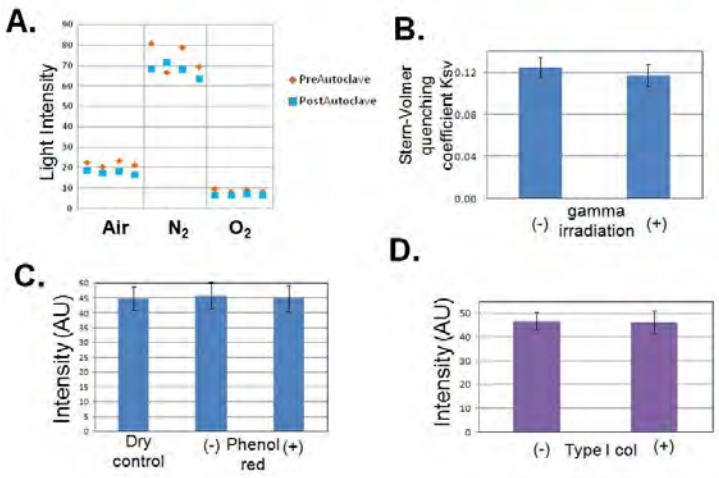

Figure 7. <u>Effects of sterilization and various cell culture compounds on Oxygen film performance</u>. A,B) Sterilization Effects. A). Effects of sterilization with autoclaving on observed fluorescence of Pt-TFPP in PS film. Degradation in performance by 17% was observed. B) Effects of sterilization with gamma-irradiation on Pt-TFPP in PS film performance. No degradation observed. C,D) Biocompatibility with chemicals and compounds. C) Effects of phenol red auto-fluorescence on observed O_2 film fluorescence; D) Effects of Type I collagen auto-fluorescence on observed O_2 film fluorescence. No measurable effects of phenol red and collagen auto-fluorescence on O_2 film were observed.

from Figure 5 the surface of the more dense PDMS formulation has never recovered completely and even after 21 day aging is still significantly below original value. Furthermore, we evaluated the diffusivity of oxygen in PDMS when plasma treated membranes were stored under water, mimicking the aqueous conditions of the BCTB when tissue or cell samples will be maintained over time. It was found that contact with water has slowed surface recovery to approximately 3 weeks. These studies are important because they provide an insight into time constrains associated with oxygen delivery and removal from the cell culture chambers as function BCTB age and fabrication materials.

Another important aspect of our thin film sensor development is its biocompatibility and possible performance degradation due to pre-culture treatments or interferences from biological samples. First we tested effects of various sterilization methods on performance of our O_2 sensing film. It was found that a typical 20 min sterilization cycle in the autoclave not just mechanically damages the film (Figure 6) but also results in a 17% reduction in film performance (Figure 7A). Gas sterilization could not be used due to PDMS permeability to gasses, which could result in toxic gas accumulation within the bulk and then possibly leaching out during the actual cell culture step. After extensive testing, we found that sterilization with gamma irradiation (a typical alternative approach)

worked extremely well. We have performed multiple tests and determined that the Stern-Volmer quenching coefficient of the film, Ksv, which serves as a measure of film sensitivity, did not changed even after overnight (8-9 h) exposure to gamma rays (Figure 7B). Additionally we have determined that auto-fluorescence of both: phenol red (media pH indicator) and type I collagen (our 3D culture matrix), had no affect (Figures 7 C and D) on fluorescence measurements of the O_2 film in the spectral range of interest (Ex = 540 nm, Em = 650 nm). During our biocompatibility studies we observed that MCF10A and their variants grew normally on top of PS film containing Pt-TFPP when cultured open-faced inside cell culture chambers placed in standard Petri dish.

B) Adapt BCTB to include control of Acidic (pH) conditions within the same BCTB cartridge. (M 2-12)

i) Utilize microfluidic perfusion system to maintain pH within each biopsy/cell chamber.

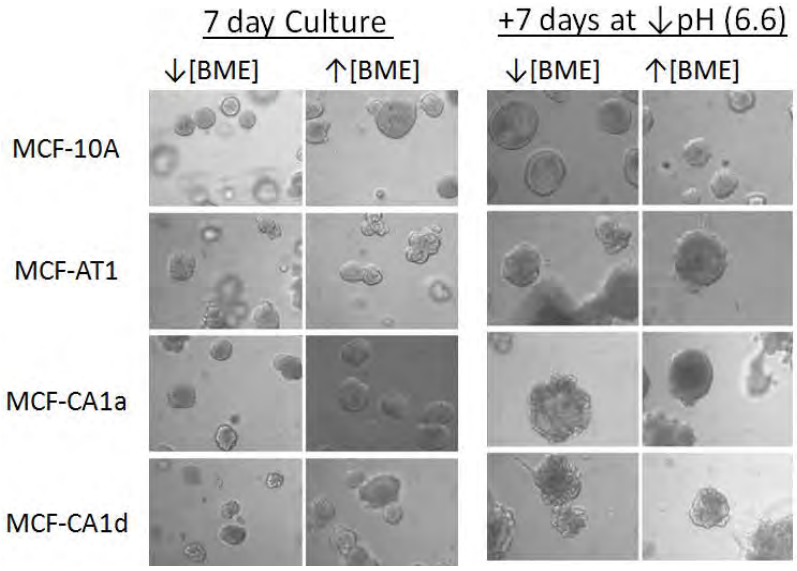

Figure 8 <u>MCF-10A, -AT1, -CA1a and -10 CA1d cell line variants</u> were cultured in the presence of standard (~3 mg/ml Matrigel) or High Concentration Basement Membrane (20 mg/ml). Cells were cultured in standard media for 7 days. At day 10, cells were transferred into either standard pH (7.4) media, or switched to low pH media (DMEM/F12 standard media + 10mM MES pH 6.6). Shown are representative images of mammosphere formation at 7 and 14 days of culture.

We used this same MCF10 variant lines and organotypic cell culture system to begin applying variations in pH within the BCTB. We are targeting "neutral" and "acidic/low" pH within our system. As we will begin to couple 3 different defined conditions within our BCTB (ECM densities, oxygen concentrations and pH levels), we compared neutral and acidic pH conditions on 2 distinct ECM densities grown under uniform, normoxic oxygen conditions. We used our MCF10A lines grown under organotypic conditions and compared mammosphere formation at 2 defined Matrigel/Basement membrane extracellular matrix densities for 7 days. Cells were seeded within 3D matrigel at low (3 mg/ml) or high (20 mg/ml) BME concentrations and mammospheres were allowed to form for 7 days using neutral pH buffered media (7.4 pH, standard media formulation). After 10 days of growth, the mammospheres cultures were transferred into DMEM/F12 standard media including 10mM MES that was 6.6 pH. Interestingly, in our preliminary findings we can see more "invasive" structures form in the low pH, high ECM growth conditions (compare figure 8 and 3). This will be used to standardize conditions within our BCTB culture system.

ii) Develop pH sensitive thin film which is chemically and optically compatible with previously developed (part A) oxygen sensitive film

Relying on our knowledge and experience gained during development of the oxygen sensitive film for 2D visualization of O_2 distribution within the BCTB, we have utilized a similar methodology to develop a pH sensitive thin film. We used a modified version of SNARF 5, SNARF®-5F 5-(and-6)-carboxylic acid, acetoxymethyl ester from Invitrogen, as a pH sensitive dye that was imbedded in Nafion film, which was spin - deposited on top of microscope slides. Initially, 50 µg of the dye were dissolved in 200 µL of a 50:50 mixture of acetone and methanol. Once the dye was dissolved, 2 mL of the 5% solution of Nafion were added to the mixture and allowed to sit for 1 h. Then, KW – 4A spinner (Chemat Technologies) was used to spin-coat 1"×2" microscope slides with 500 nm thick Nafion film containing pH dye. It was found that overnight stabilization of the film significantly improved its mechanical stability and adhesion to the glass substrate. Figure 9 A and B show spatial changes in fluorescence of the pH film as a function of two buffers with pH 6 and 8 flowing through the microfluidic channel placed on top of it. During our initial tests we have also found that the optical properties of the dye immobilized within the Nafion matrix have slightly changed as compared to it being in free solution. Figure 9 C shows the fluorescence spectra of the 500 nm thick Nafion film as a function of the buffer pH in contact with the film. As expected the fluorescence intensity in the yellow - orange region (outlined by the dotted box) was inversely proportional to the pH of the buffer. However, the pH dependent red shift in intensity (expected in free solution) was not observed as well as the pH-independent isosbestic point at 610 nm. Currently, we are further investigating optical properties of the film, sterilization methods, and calibration protocols.

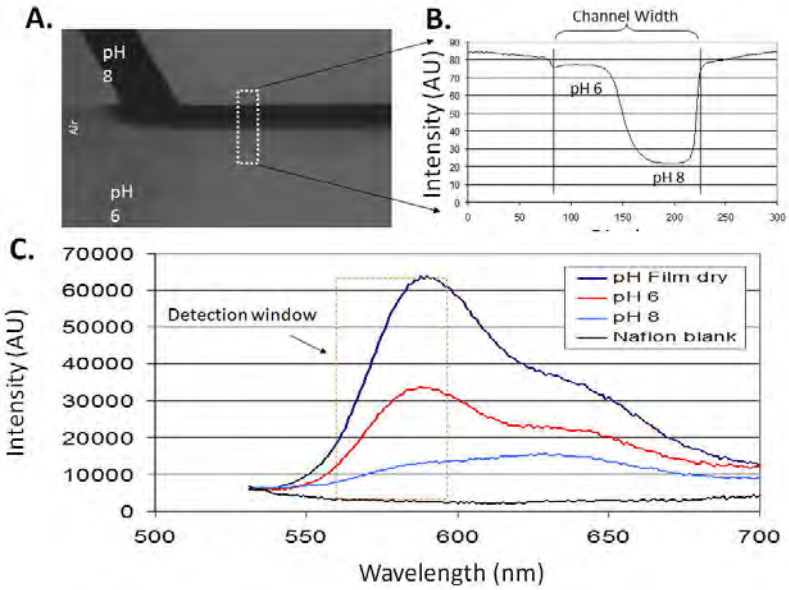

Figure 9. <u>Optical response of the SNARF – Nafion pH sensitive film in presence of different pH calibration buffers.</u> A) Observed changes in fluorescence of the pH film in response to pH 6 and pH 8 buffers flowing through the 100 mm wide microfruidic channel (Ex = 488 nm, Em = 575 / 40 nm). B) Line profile of the observed fluorescence signal across microfuidic channel as two buffers flow on top of pH sensitive film (40 pixel average along channel length). C) Measured emission spectra of the pH sensitive film in: absence of fluid contact (native fluorescence); in presence of buffers with pH 6 and 8; and auto-fluorescence of the Nafion film without SNARF dye.

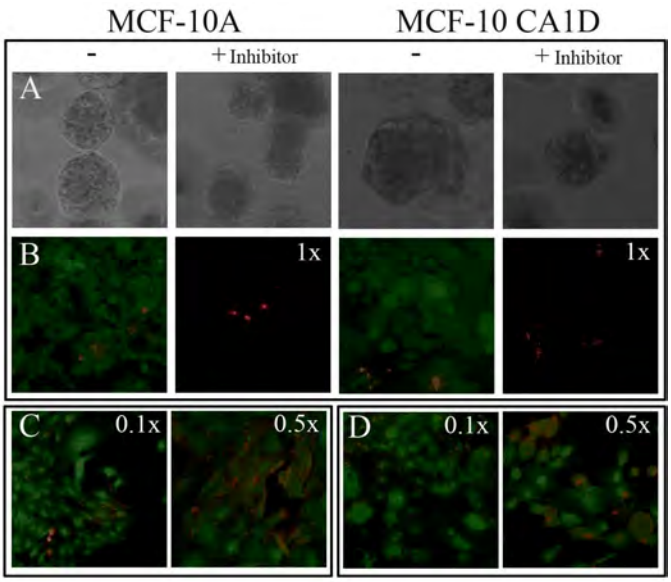

Figure 10: **MCF-10A and -10 CA1d** were cultured in the presence or absence of combination proteinase inhibitor cocktail and evaluated for A) mammosphere formation in 3D matrigel or B-D) Dose dependent toxicity to increasing amounts of proteinase inhibitor treatment affecting viability as evaluated by Live-Dead Viability Assay at 1µM of Calcein AM (Green=live) and 2µM of Ethidium homodimer-1 (Red=Dead). Inhibitor treatment in (A) used drug concentration that was not toxic to cells.

(Aprotinin, for serine; GM6001, for metallo-; E-64, for cysteine; and Pepstain A, for aspartic; Calbiochem). As we wanted to demonstrate long term delivery of drug into our system, we first determined the amount of drug that could be added to traditional cell culture without affecting cell viability. Viability was assessed using Live/Dead Assay (Molecular probes). This was applied to monolayer cell cultures in a incrementally lower concentrations and cell viability was evaluated. The full dose was toxic to cells within 48 hours of application. However, lower concentrations did not affect cell viability.

Next we assessed whether lowering the dose would still be affective as a extracellular matrix inhibitor. For 3D cell cultures, mammospheres were allowed to establish in standard cell chamber slides and then transferred into the BCTB into new 3D matrix containing fluorogenic probes(18;19). The spheroids were cultured in the presence and

Task 3. To determine whether drug response of a tumor biopsy sample evaluated within the BCTB predicts responsiveness in vivo using murine models of breast cancer.

As a first step towards validation of drug responsiveness in our BCTB, we evaluated the ability of a known inhibitor to blunt cellular growth in our reactor system in a side-by-side comparison of traditional cell culture techniques. The MCF10A human mammary epithelial cell line and their invasive were used to validate drug delivery into the bioreactor. Cell lines were cultured in low density 3D matrigel conditions.

Mammoshere formation is dependent upon a number of cellular processes and is regulated by extracellular proteinase activity(16;17). A Matrix protease inhibitor cocktail shown to target a broad spectrum of extracellular matrix proteinase activity was used at known concentrations that affect migration

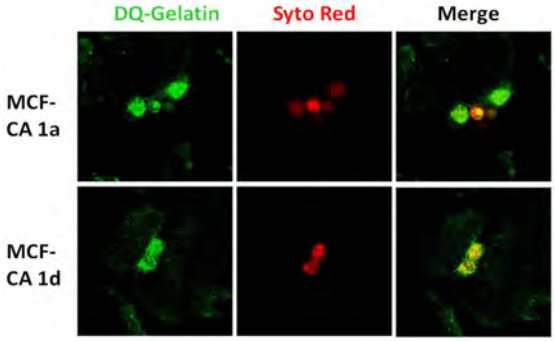

Figure 11. **Confocal microscopy of MCF cell variants suspended in 3D matrix** containing fluorogenic protease activity probe. Cells were suspended in matrix gelled with 2 mg/ml fluorogenic probe. Following 24 hr, protease activity was detected by an increase of fluorescein fluorescence due to proteolysis of DQ-gelatin (Ex/Em 495/515 nm; Molecular Probes). Cells were labeled with Syto Red for visualization (Ex/Em 615/632 nm; Molecular Probes). Images representative of one plane of a z-stack (40x).

absence of Matrix proteinase inhibitors for 24-48 hours. Protease activity will be measured as an increase of fluorescein fluorescence of matrix embedded DQ-gelatin using confocal microscopy (Ex/Em 495/515 nm; Molecular Probes; (20-22)) (Fig. 11). Changes in fluorescent intensity was analyzed morphometrically with Metamorph Software. We able to demonstrate effective protease inhibition using these reduced concentrations of the proteinase inhibitor cocktail.

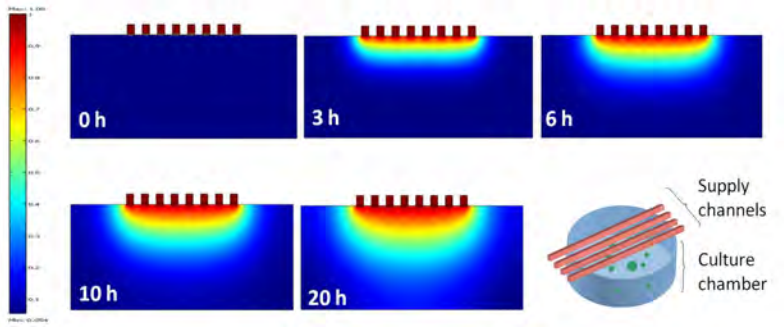

Figure 12. **Preliminary diffusional model of drug delivery into the BCTB filled with collagen.** Supply channels are 100 by 100 µm, culture chamber is 1 by 3 mm and a large molecule "drug" with a diffusion coeffient D = 2.75e-12 m^2/s (~2 MDa fitc-labeled dextran through collagen gels). Lower Left Panel: 3-D view of the Cell culture chamber used in the initial modeling efforts with only 4 channels pictured. Actual devices have 8 supply channels.

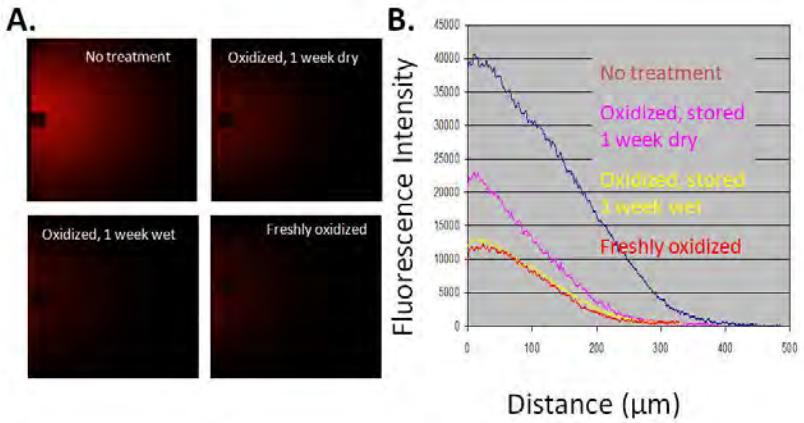

Figure 13. Partition of small molecules into PDMS as a function of surface properties and storage conditions. (A) Cross-sectional view of the rhodamine B diffusing into bulk PDMS observed after 24 h incubation period. (B) Intensity profiles corresponding to fluorescence images in (A). Note: Absence of any surface treatment can result in a high degree of adsorption of small molecules to the surface, which can lead to unpredictable changes in local chemokine concentrations. This could have a pronounced effect during long-term culture or when total volumes of handled media is on the order of several mL or nL.

We also needed to assess the ability to deliver drug within the culture chamber of the BCTB. To begin to address this issue, alongside of our fabrication effort, we have developed a computational model of the reactors using COMSOL Multiphysics software package (COMSOL Inc, Burlington, MA). Shown in Figure 12 is the preliminary 3D model illustrating diffusion-based delivery of a large molecule drug with a diffusion coeffient D = 2.75×10^{-12} m^2/s into the BCTB cell culture chamber containing collagen(72). This model can be easily adapted to include various matrix stiffnesses and nutrient transport properties.

In order to address possible concerns that some molecules could potentially adsorb to the surface of the cell culture chamber and thus potentially alter the actual concentration delivered, we have performed a series of experiments looking into rhodamine B partitioning into PDMS as a function of surface treatments. As it could be seen from Figure

13, in absence of any oxygen plasma treatments rhodamine easily partitions into PDMS and diffuses into the bulk of the material, while plasma treatment creates a silica barrier that significantly impedes such a behavior. This barrier remains effective even after week long storage under wet conditions. Actually, these results are in excellent agreement with the O_2 diffusion experiments shown in Figure 5, where surface oxidation created a barrier for the oxygen molecule to diffuse into PDMS bulk.

Drug delivery was accessed by introducing matrix protease inhibitors into the system. In vitro acinar development was correlated with pharmacological down-regulation of matrix proteinase activity in these cell systems. We cultured cells in the absence or constant presence of the protease inhibitor cocktails shown to target a broad spectrum of extracellular matrix proteinase activity with minimal changes to cell viability. As shown in figure 14, this protease inhibitor cocktail down-regulated morphogenesis of the MCF-10A variants within the BCTB.

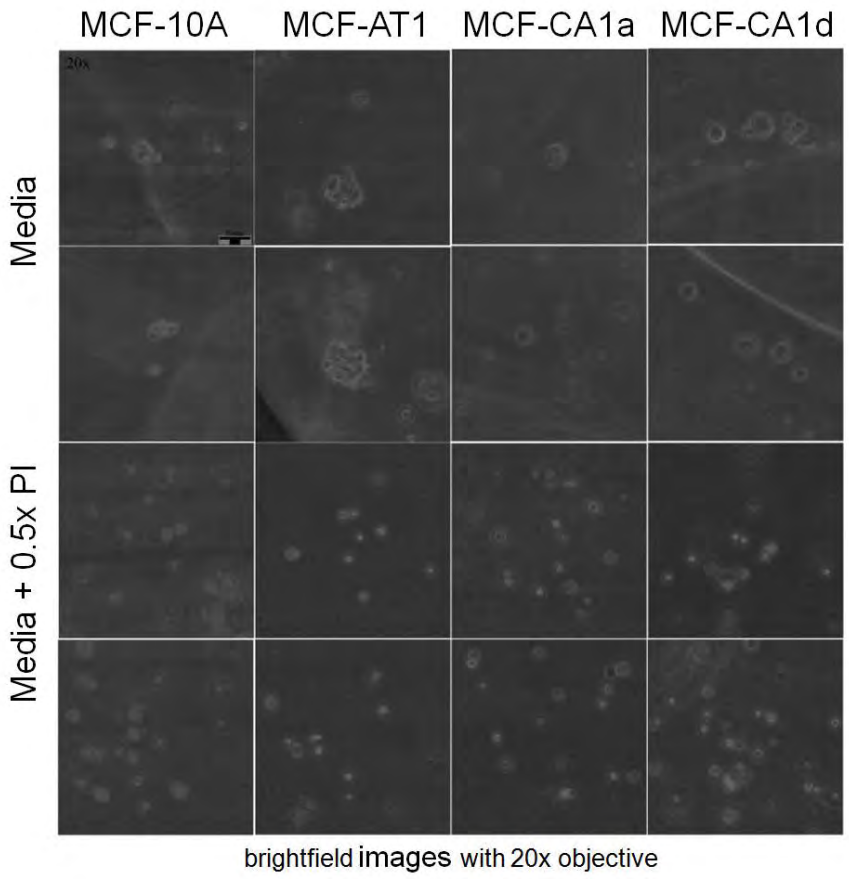

Figure 14. **<u>Proteinase Inhibitor treatment of various MCF10A cell variants</u>** demonstrating a significant reduction in the size of mammospheres cultured with TTB. The protease inhibitor cocktail is defined in the Methods. Images were taken with the TTB being fully assembled and continuously perfused as shown in Figure 2 C and D.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- BCTB Modular design: each reactor can be maintained under different flow (nutrient exchange) profile or connected to a single supply source via manifolds
- Manifolds in combination with gravity induced flow are effective low cost approach to passively regulate the perfusion rates through BCTB
- System is capable to maintain long-tern (>20 days) cellular viability and allows for proper formation of mammospheres
- System allows for efficient delivery of a treatment as was confirmed by inhibition of mammosphere formation and proteinase activity
- There is a significant difference between diffusion coefficients of plasma treated and native PDMS
- After 3 day aging, the diffusion coefficient of O_2 in PDMS is restored close to the original, untreated value when maintained in air. If the surface remains in contact with aqueous solutions (culture media) the recovery rate is significantly reduced (3 weeks). Even though highly cross-linked PDMS has the same permeability in the untreated state, it remains significantly less permeable to O_2 even 3 weeks after the plasma treatments.
- Oxygen and pH sensitive film for 2D mapping of O_2 and pH distribution, respectively, within the BCTB have been developed. Oxygen Sensitive film is compatible with standard cell culture and gamma ray sterilization methods.

REPORTABLE OUTCOMES:

Meeting Report

1. E.M. Lillie, Markov D.A., Garbett SP and McCawley LJ, Comparing diffusion coefficients through thin PDMS membranes exposed to various surface treatments, BMES 2010, Austin, TX, 2010.

Employment/Research Opportunity.

Dr. Dmitry Markov, an electrical engineer by training, was hired into a non-tenure track faculty appointment of Research Instructor within the Department of Cancer Biology, Vanderbilt Univ Med Ctr in part due to work related experience in Breast Cancer and application of novel technologies towards understanding this disease process afforded by the research opportunity presented with this award.

CONCLUSION:

As a major investment towards our overall goal, we focused on developing a capillary perfused bioreactor capable of sufficient nutrient supply and metabolite exchange for thick tissue culture in microfluidic environment and that could deliver an inhibitor of a cellular process that would impact cellular growth.. We developed a drug delivery regimen and applied this within our portable breast cancer tissue bioreactor (BCTB) that is capable of maintaining sterile microenvironment and sustaining cell growth, maturation, and organ formation during three week long cell culture. The performance of drug delivery within the BCTB was validated with MCF10A cell line and its tumorigenic variants that have developed into hollow lumen spheroids in 21 days. Real-time staining and visualization of fully developed mammospheres was possible within the closed bioreactor system. Fully assembled TTB was compatible with regular and fluorescent microscopy and allowed for optical assessment of the mammospheres development and proteinase activity using conflocal microscopy. We applied a proteinase inhibitor regimen and could monitor the concomitant inhibition of mammosphere morphogenesis.

We have developed thin film sensors to monitor oxygen states within the fully developed BCTB. We have characterized oxygen diffusion through the PDMS under various surface treatments (i.e., plasma, water storage) and stiffnesses and have generated rules for our gas delivery system that is within the PDMS parallel to our cell growth chambers. We have also characterized the effects of common sterilization techniques on our thin film integrity and feel that we have a good method currently to begin to move forward on joining our oxygenation sensing to our BCTB. Furthermore, we have developed and testing thin film sensors to monitor pH states within the fully developed BCTB.

Our goal is to engineer a Breast Cancer Tissue Bioreactor will maintain the in vivo functionality a patient's tumor biopsy sample ex vivo and be used to screen response to anti-tumor agents, such that the patient's own tumor tissue can be used to predict that best therapeutic treatment option for that individual patient. The conditions in the tumor microenvironment affect cellular functionality of the cell types within, including the effectiveness and response to anti-cancer therapeutics. Thus, we believe that the predictability of the patient's response to chemo- or molecular therapeutics will require an exact mimicry of the in vivo tumor microenvironmental conditions. The purpose of the devices and technologies described herein is to perfect a novel small scale bioreactor that allows unprecedented control over specifying the exact environmental conditions to maintain a breast tumor biopsy sample, thus allowing recapitulation ex vivo of the typical tumor microenvironment. We have laid down a foundation and design rules for future reactor developments as we implement the BCTB with controlled oxygenation and pH states for maintenance of tumor biopsy samples. It would be beneficial to develop a microfluidic device that would be compatible with 3D organotypic cell culture and capable of maintaining tumor biopsy specimen for extended periods of time in its natural 3D microenvironment allowing for testing of various treatment options in order to select the most optimum for the patient.

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